Untangling the Mystery of Alzheimer's Disease

Understanding Molecular Mechanisms for Novel Therapeutic Approaches

Sovan Sarkar, Anupam Choudhury and T N Avinash

Alzheimer's disease is one of the most common neurodegenerative disorders amongst the elderly, leading to dementia. Current studies of its molecular basis reveal various factors culminating in the cytotoxic cascade associated with the disease. Progress in understanding these events has led to the identification of novel therapeutic targets. This article is an attempt to understand the global scientific efforts in deciphering the mystery of this disease for a possible cure.

Imagine yourself to be in your sixties. Old age brings its own set of maladies, and so you ignore a trivial matter of forgetting where you kept the car keys. Gradually, you become more absent-minded, and lose interest in all your hobbies – be it chess, golf or literature. Later, you have difficulties in recognizing people, even close relatives. Towards the later stages, you cannot do without assistance, even for routine tasks like eating, bathing, dressing or returning home. In the final stage, you become mute, incontinent and bedridden, and finally die of other illness. Frightening, isn't it?

This scenario seems to be right out of the pages of a science fiction horror story, but sadly, it is reality for people suffering from Alzheimer's disease (after the German neurologist Alois Alzheimer). Alzheimer's disease (AD) is the most common cause of senile dementia (*Box* 1) and is estimated to affect 22 million people worldwide by the year 2025 and other age-related neurodegenerative disorders leading to dementia are on the rise because of increase in life expectancy and decrease in human mortality.

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Sovan Sarkar's research interests are gene therapy, apoptosis in cancer treatment and therapeutic approaches for neurodegenerative disorders.

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Box 1: Dementia

The term 'dementia' refers to several illnesses, which affect the functioning of the brain, leading to disruptions in memory, reasoning and emotional stability. 'Senile dementias' are clinical syndromes affecting the aged, and show two abnormalities: memory loss in an otherwise alert individual; and impairment in at least one other area of cognition – language, problem solving, judgement, calculation, attention, perception, etc. The chief cause of senile dementia is Alzheimer's disease, followed next by cerebrovascular disease (alone, or, in combination with AD). In India, dementia is commonly associated with cerebrovascular disease. Other causes include Lewy body dementia, Parkinson's disease, fronto-temporal dementia, alcoholism, drug-abuse, brain tumour, infections like HIV, syphilis, etc.

Abbreviations used:

AD: Alzheimer's disease
βAPP: β-Amyloid precursor protein
Aβ: β-Amyloid
NFT: Neurofibrillary tangles
PHF: Paired helical filament;
τ: Tau protein.

Clinical Manifestations of Alzheimer's Disease

It is now clear that AD is a multifactorial syndrome rather than a single disease. The pathological hallmarks of this disease are the amyloid and senile plaques (which appear earlier on), and the development of neurofibrillar tangles (NFT). Amyloid plaques (which resembles bomb craters) are spherical, multicellular lesions usually found in the limbic and associated neocortex areas. Amyloid-β-protein, which is a major component of both amyloid and senile plaques, is deposited in the extracellular space as a fluffy material. Senile plaque is surrounded by neurites (axon/dendrites) and invaded by microglia and astrocytes – the inflammatory cells of the brain. These are found in the frontal cortex, hippocampus, amygdala, occipital cortex, thalamus, and less frequently in basal ganglia and cerebellum. On the other hand, NFTs are intraneuronal lesions consisting of non-membrane bound bundles of ~10 nm. paired helical filaments, which are seen in later stages of the disease. NFTs appear to be more important for the altered neuronal function than plaques. The presence of NFTs in the frontal cortex is highly suggestive of AD. (*Figure* 1)

Keywords
Neurodegenerative disorder, senile dementia, β-amyloid plaques, neurofibrillary tangles.

Amyloid Plaques: Protein Processing going Haywire

The amyloid plaques (*Figure* 2) are composed almost exclusively of a small peptide called β-amyloid (Aβ), which is derived from a larger integral membrane protein called β-amyloid precursor

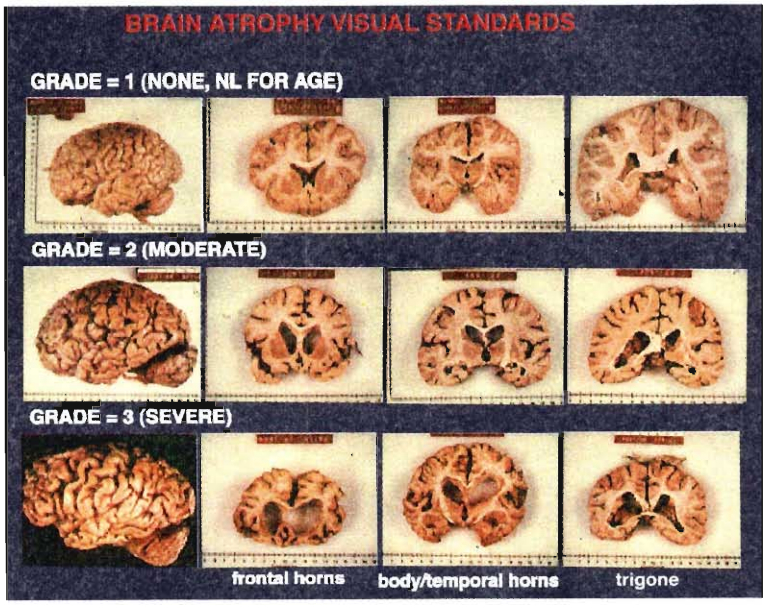


Figure 1. The 12 panel photographic array represents the visual standards based scoring system for cerebral atrophy in brains from aging nondemented control and Alzheimer-dementia subjects at autopsy.

protein (βAPP). Biochemical analysis on how βAPP is processed revealed the generation of the toxic Aβ peptide (*Figure* 3). Some of the βAPP in the membrane are internalized and degraded. A small subset of the APP molecules is found to be degraded initially by α-secretase enzyme (a metalloprotease) to produce an 83-residue COOH-terminal fragment (C83) and a soluble version of βAPP (α-APPs), and then by γ-secretase enzyme (resembling aspartyl protease) to give rise to a harmless peptide of 3 kDa called p3. However, when βAPP is cleaved initially by β-secretase enzyme (resembling aspartyl protease) to produce a 99-residue COOH-terminal fragment (C99) and βAPPs, followed by γ-secretase cleavage, either a 40 amino acids Aβ peptide (Aβ_{40}) or a 42 amino acids Aβ peptide (Aβ_{42}) of 4 kDa is formed. The more toxic version is Aβ_{42} as it is hydrophobic and rapidly forms aggregates in the extracellular space.

Figure 2. β-amyloid plaques as seen in cerebral cortex of a subject with advanced Alzheimer's disease.

More insights were gained from genetic analysis. Mutations in the βAPP gene, located on chromosome 21, that interfered with the normal degradation of the protein, i.e., modifications in regions cleaved by α-secretase(s) or γ-secretase(s), resulted in earlier onset of the disease as more Aβ_{42} was being formed. Soon,

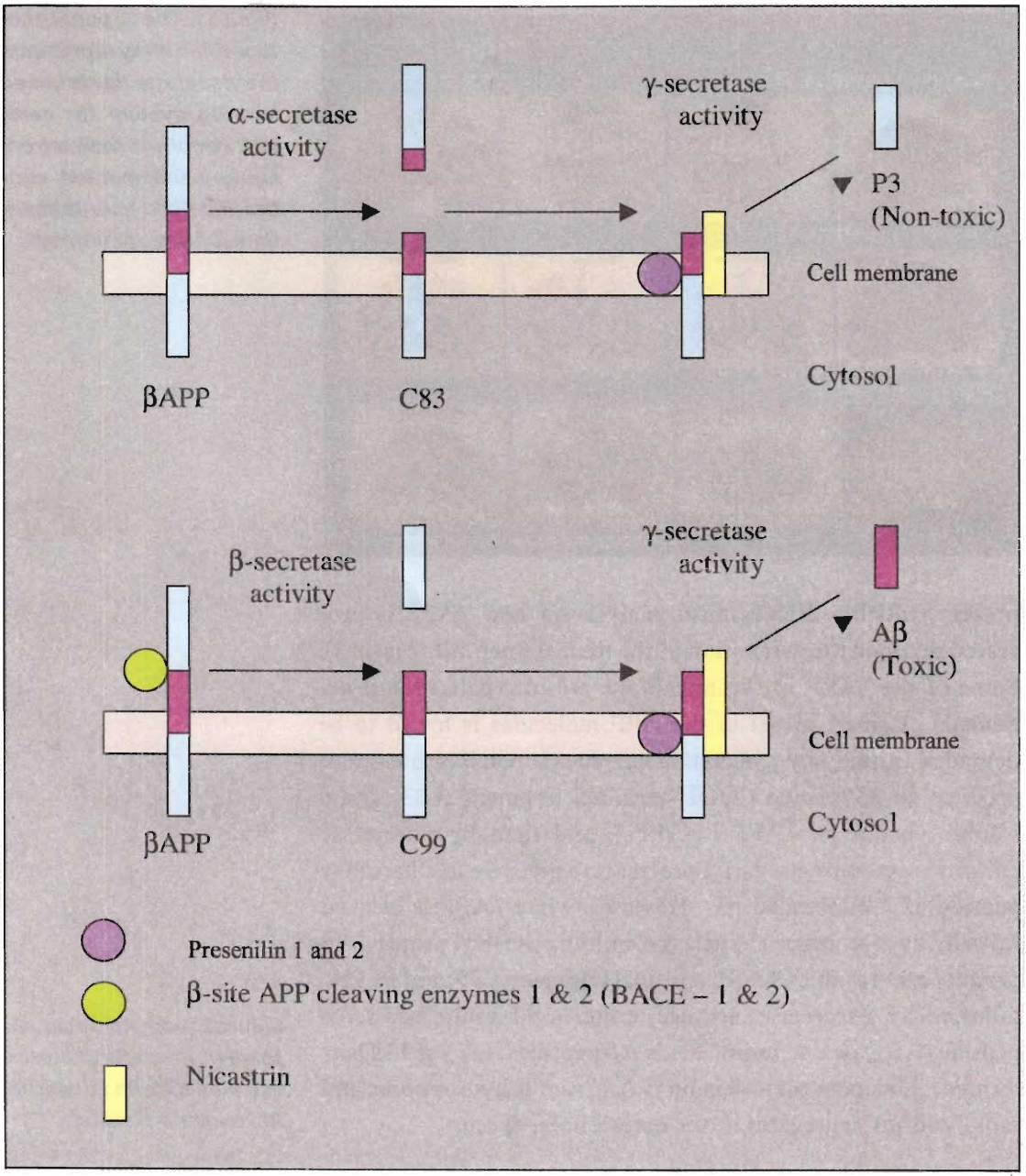


Figure 3. βAPP processing and generation of toxic $A\beta_{42}$.

three more genes were implicated in the development of AD. Two of them, *Presenilin* 1 and *Presenilin* 2 located on chromosome 14 and 1, respectively, produce similar transmembrane proteins, which are essential cell fate determinants. Mutations

in these genes were found to increase the amounts of $A\beta_{42}$ produced, and it was postulated that they might be γ-secretase itself. Recently, it has been reported that *Presenilin*, in concert with another protein nicastrin, is responsible for the γ-secretase activity. The gene for β-secretase is located on chromosome 11, but no AD-causing mutation in this gene has been identified so far. A β-secretase homologue, BACE2, located on chromosome 21, indicates the possibility that this protease contributes to AD-associated with Down's syndrome. However, its presence in very low amount in brain suggests that the AD associated with Down's syndrome is probably due to the presence of an extra copy of the *APP* gene, which is also present on chromosome 21.

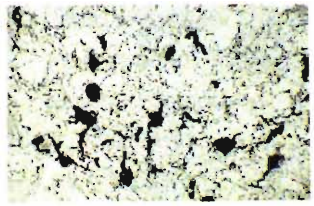
Polymorphism in the *APO*E gene on chromosome 19 was the next to be identified as a risk factor for AD. Apolipoprotein E (ApoE) is involved in transporting cholesterol and other lipids in the blood. The gene occurs in three allelic forms designated as $\varepsilon 2$, $\varepsilon 3$ and $\varepsilon 4$. The most common form is $\varepsilon 3$, which confers protection against development against AD, whereas the individuals with *apo*$\varepsilon 4$ alleles face an increased risk in developing AD. Apoe4 not only promotes fibrillogenesis by the amyloid peptides, but also itself is capable of forming fibrillary proteins.

In a nutshell, what has been reported are that mutations of genes on chromosomes 1, 14 and 21 result in early onset of AD, which are inherited in a Mendelian pattern. In addition to these inherited cases, mutations in the apolipoprotein gene on chromosome 19 make the individual more susceptible for developing AD in older age.

It takes Tau to Tangle!

Similar biochemical analysis revealed that the neurofibrillar tangles (*Figure* 4) consisting of paired helical filaments were formed of hyperphosphorylated versions of a microtubule-binding protein called tau (τ). Microtubules form the cytoskeleton and are involved in various cellular processes including spindle

Figure 4. Typical hippocampal neurofibrillary tangles (NFT) occurring in Alzheimer's disease.



body formation during cell division, and transport of various vesicles, protein assemblies, etc. in a cell. Intracellular tau gets phosphorylated by microtubule-associated protein kinases or glycogen synthase kinase (GSK3) and is deposited on the cytoskeletal filaments. Thus it promotes formation of intracellular NFTs. In addition to neurofilaments and tau, NFTs also have ubiquitin as a component, but the function of each in the formation and stabilization is still unclear. The disruption in the function of tau leads to collapse of the microtubule skeleton, thus disrupting the vital activities of a cell.

But how does tau relate to $A\beta_{42}$? All evidence so far seem to indicate that tau hyperphosphorylation is a cytological effect of $A\beta_{42}$. The best evidence for this is from patients with frontotemporal dementia. Such people have mutant tau protein and NFTs are found quite early, although no amyloid plaques are seen. The cascade of events that cause tau hyperphosphorylation by $A\beta_{42}$ is yet to be determined.

How do the Neurons die?

The $A\beta_{42}$ peptide causes neuronal degeneration and death by initiating a complex cascade of events that are slowly coming into light (*Figure* 5). Aggregation of $A\beta_{42}$ in the inter-neuronal space causes a multi-prong inflammatory response involving microglia and astrocytes. Small diffusible $A\beta$ oligomers called $A\beta$-derived diffusible ligands (ADDL) have also been found to cause cell death. Extensive cytoskeletal disruption resulting from accumulation of paired helical filaments (PHFs), in association with severe disruption of axonal transport and cell metabolism, lead to accelerated neuronal death.

Aggregation of $A\beta_{42}$ in the inter-neuronal space causes a multi-prong inflammatory response involving microglia and astrocytes.

The $A\beta_{42}$ peptide binds strongly to metal ions like copper and iron, catalyzing the formation of superoxide (O_2^{2-}) from hydrogen peroxide (H_2O_2). The free radicals thus generated, coupled with the inflammatory reactions initiate a further cytotoxic cascade of events including mitochondrial disruption, ultimately leading to cell death.

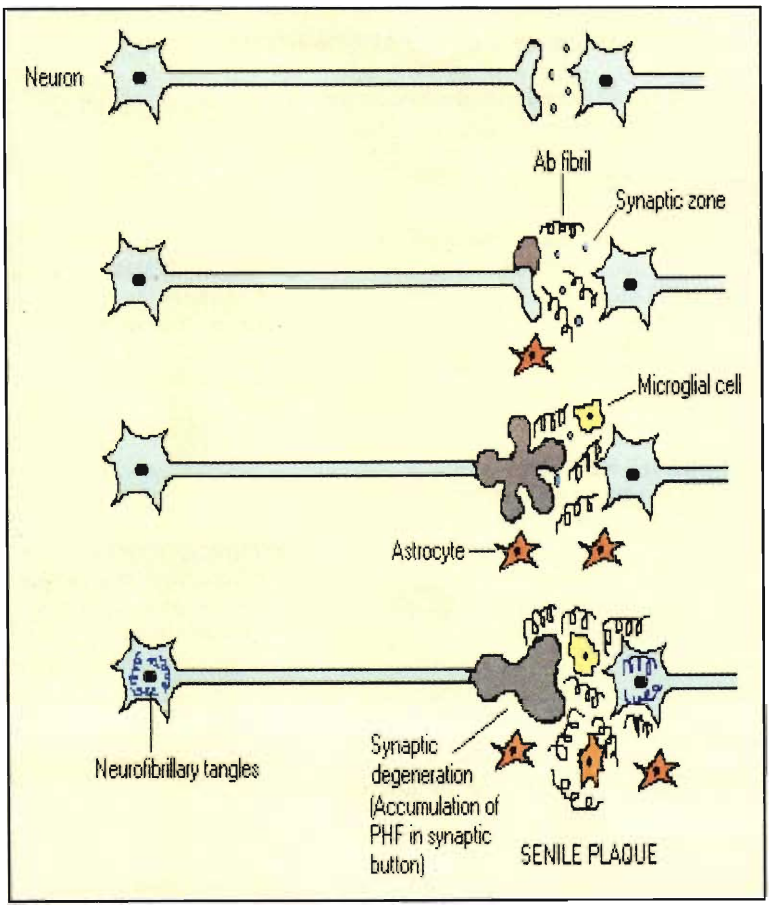


Figure 5. Schematic illustration of neuronal degeneration. Aggregation of amyloid beta protein in the synaptic zone elicits inflammatory response, followed by formation of neurofibrillary tangles. Initially, the fluffy amyloid beta protein is deposited in the extracellular space as round balls (diffuse plaques). The axons then show coiling and bending, which along with the broken dendrites entangle to form the enuritic plaque. Finally, the amyloid protein condenses as a central core around which the neurites are seen (mature plaque). At advanced stages of the disease, more of mature plaques are seen.

Recent research has shown that βAPP is also cleaved by caspases (enzymes crucial in driving programmed cell death or apoptosis) to produce another toxic fragment called C31. It is not known whether Aβ aggregation activates caspases or whether they constitute an independent death cascade. (*Figure* 6) (*Box* 2)

Therapeutic Advancements: New Hopes

Progress in understanding the neurotoxic and inflammatory cascade has currently identified specific therapeutic targets for the amelioration of human suffering. Research advancements in biochemical pathology and molecular genetics during the last decade have laid the foundation in this direction. Molecular insights into βAPP and Aβ have provided considerable information for designing drugs that will either block the formation of

Box 2: Overview of Alzheimer's Disease.

How the risk factors affect vulnerable neurons, including cytopathological responses, leading to characteristic clinical signs.

RISK FACTORS

- Age
- Presenilin 1 and 2 mutations
- *APP* gene mutation
- *apo*E allele

CLINICAL SIGNS

* Memory loss
* Cognitive defects

VULNERABLE NEURONS

- Monoaminergic systems
- Cholinergic systems

Hippocampus
Entorhinal complex
Neocortex

CYTOPATHOLOGY

* Neurofibrillary tangles
* Aβ peptide deposition (Senile plaque)
* Neuronal death

Figure 6. General progression of Alzheimer's disease.

APP, *PS*1 and *PS*2 gene mutations in the neuronal DNA

Altered proteolysis of *APP* leading to increased production of Aβ_{42} in extracellular space

Aβ_{42} forms fibrillar aggregates in the interneuronal space forming amyloid plaques

Inflammatory response (complement-mediated) leading to neuritic injury in plaques

Disruption of ionic homeostasis (Ca^{++} influx); oxidative injury due to free radicals

Altered activities in cell; tau hyperphosphorylation promotes formation of NFTs

Widespread neuronal dysfunction; death of neurons in limbic and cortical areas

Dementia of Alzheimer's disease

Box 3: Targets forTreatment

Several discrete targets for treatment in delineating the Alzheimer's disease cascade include:

- Inhibitors of Aβ production, i.e., blockers of β- and γ-secretase activity.
- Inhibitors of Aβ oligomerization or fibrillization.
- Anti-inflammatory drugs that can interfere with aspects of the microglial and astrocytic responses in the brain.
- Antioxidants, free-radical scavengers, calcium-channel blockers and modulators of signal transduction that can protect neurons from the downstream effects of accumulation of Aβ.
- Neurorestorative factors that can rescue synapses and cell bodies undergoing injury.

the toxic protein $A\beta_{42}$, or alleviate the peptide's effect once it has been formed. (*Box* 3)

Till date, there is no treatment available for complete cure of Alzheimer's disease. The drugs that are currently used for the management of AD are Donepezil (Aricept) and Tacrine (Cognex), both of which are acetylcholinesterase inhibitors. Thus they prevent synaptic degradation of the neurotransmitter acetylcholine and thereby act as memory enhancers.

Two inhibitors of β-secretase, OM991 and OM992, have been recently developed to block the formation of $A\beta_{42}$. A recent report suggests the identification of low molecular weight chemically stable fenchylamine sulphonamides as *in vitro* inhibitors of γ-secretase. These compounds are paving the pathway for efficient inhibition of Aβ formation.

Currently, efforts are under way to reduce the deposition of Aβ by using compounds that mimic dyes such as Congo red. These compounds can insert into amyloid plaques and break down the aggregations of Aβ from within.

Recent studies suggest that the oxidative damage in the neocortex associated with AD is a result of gradual build-up of metal ions like zinc, and more significantly copper. Metal chelators that chelate copper and zinc, thereby diminishing the oxidative burden are currently under investigation.

Neuroprotection is required by the neurons under stress and are therefore vulnerable.

The role of H_3-receptor (histamine receptor subtype) in learning and memory indicates that H_3-receptor antagonists can have a role in the treatment of memory disorders such as AD. Perceptin, a potent and selective H_3-receptor antagonist, has been devised in this aspect. Moreover, approximately 45% of glutamate receptor in the human brain respond to DL-α-hydroxyl-5-methyl-4-isoxazole prepionic acid (AMPA) as an agonist, and are consequently designated AMPA type receptors. These have been implicated in memory and other higher order cognitive function such as thinking. AMPA receptor modulating agents such as CX516 have been shown to enhance memory in rats and normal elderly volunteers. Galantamine therapy, where cholinesterase is inhibited, shows sustained cognitive benefits for AD patients. The present findings confirm that huperzine A can alleviate the cognitive dysfunction induced by intracerebroventricular infusion of β-amyloid protein in rats.

Neuroprotection is required by the neurons under stress and are therefore vulnerable. This approach is targeted to slow down disease progression as opposed to improving cognitive function. In this class of agents are antioxidants such as vitamin E, selegiline, ascorbic acid and *Ginkgo biloba* extracts. Studies on anti-inflammatory agents like indomethacin for delaying the progression of AD is on. Since chronic inflammation occurs in AD pathogenesis and lipoxygenases are important mediators of inflammatory processes, the 12-lipoxygenase inhibitor baicalein is recently reported to attenuate neuronal apoptosis.

The potential role of estrogen for alleviating memory problems relating to AD is also under investigation. Estrogen can synthesize acetylcholine, increases formation of synapses and also numbers of nerve growth factor receptors in those regions of brain vital for memory and cognitive functions. (*Figure* 7)

Amyloid β Vaccination

The remarkable discovery of a vaccine that can curb memory loss in AD in a genetically engineered mouse model of the

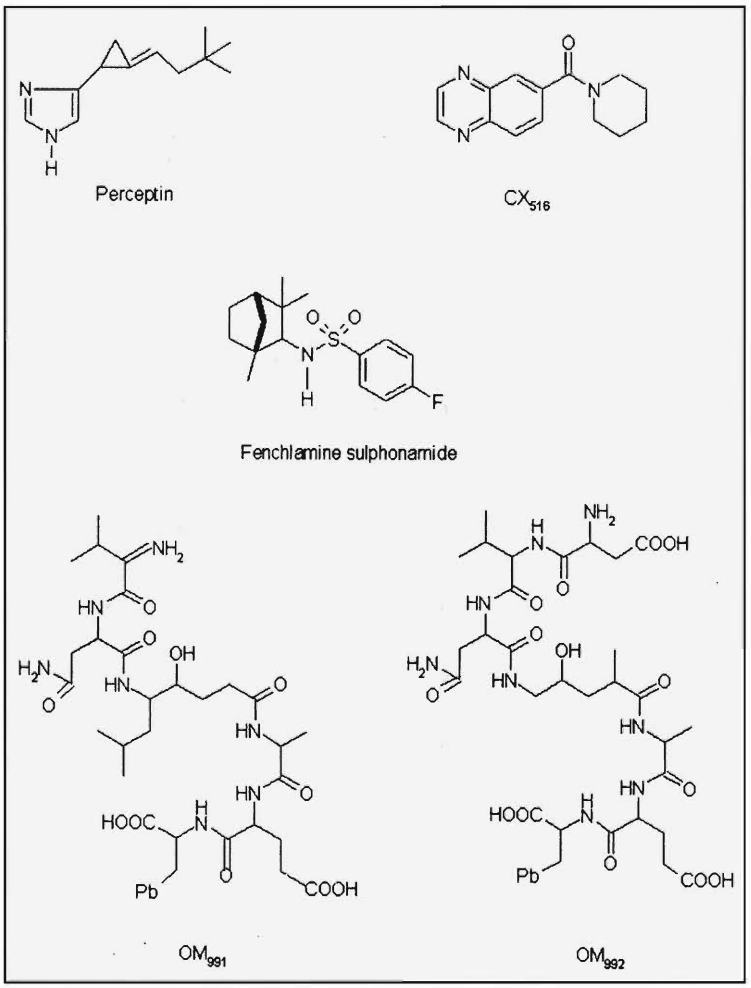


Figure 7. Various drugs in phase II clinical trials for treatment of Alzheimer's disease.

disease may eventually be used to prevent and treat this disease in humans. Immunization with amyloid-β-protein (Aβ) reduced learning and memory impairments in AD transgenic mice. The number of plaques, however, was reduced in vaccinated mice. Such mice also developed antibodies against Aβ, which provide impetus for the exploration of antibodies against Aβ as a means to prevent or treat AD.

Neural Stem Cell Therapy: A Future Possibility

The fact that neural stem cells (immature cells in the brain that have not yet fully formed) can give rise to any cell found in the

Gene therapy has emerged as new hope for Alzheimer's patients, with human clinical trials currently under way.

nervous system show a ray of hope for repair of damaged brain. Neural stem cells can be manipulated, as shown experimentally, to deliver therapeutic genes to damaged brain areas as well as replenish cells missing in genetic and acquired brain disorders.

Transplantation of neural stem cells has been reported in animal models. Once in place, these cells develop into oligodendrocytes that make the myelin sheath. Myelin sheath insulates nerve fibres and enables efficient impulse conduction. These cells when transplanted into newborn rodents having genetic defect in their ability to produce myelin can make myelin. Also, neuronal regeneration in adult human cortex proved that the mature brain might harbour neural stem cells. Studies have shown such regeneration in hippocampus, a region involved in memory. This may be useful in harnessing the neurons latent regenerative powers to repair injuries and recover from disease. Treatments of neurodegenerative diseases, such as AD, will some day be true in future.

Gene Therapy: Another Ray of Hope

Gene therapy has emerged as new hope for Alzheimer's patients, with human clinical trials currently under way. In this approach, fibroblasts have been genetically modified to produce nerve growth factor (NGF), which prevents neuronal death and enhance the function of the remaining brain cells. These modified fibroblasts have been implanted at different locations in the brain.

Conclusion

Alhough our understanding of the molecular basis of Alzheimer's disease has increased considerably in the recent past, many pieces of this complex puzzle are yet to be elucidated. Putting together these pieces is a more daunting task than imagined. Hopefully, a better understanding of the disease will not only lead to its therapeutic advancements, but also may facilitate the same for other neurodegenerative disorders like Parkinson's disease and Huntington's disease.

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Inspired by Marvin Minsky, Shannon built the 'Ultimate Machine'. It is a small box, with a switch on the side. When you switch it on, the lid rises, a hand emerges, reaches down, turns the switch off and retreats into the box. The lid closes, leaving one embarassed at having disturbed the machine's peace!